ACS Macro Letters

Viewpoint

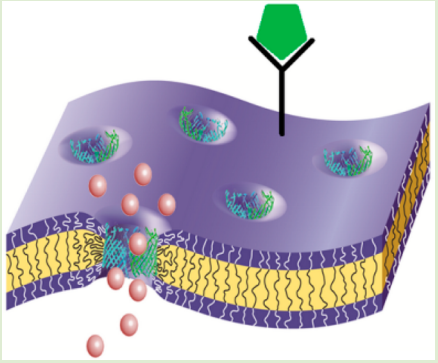
pubs.acs.org/macroletters

Planar Biomimetic Membranes Based on Amphiphilic Block Copolymers

Justyna Kowal,[†] Xiaoyan Zhang,[†] Ionel Adrian Dinu, Cornelia G. Palivan,* and Wolfgang Meier*

Chemistry Department, University of Basel, Klingelbergstrasse 80, CH-4056 Basel, Switzerland

ABSTRACT: Planar biomimetic membranes based on amphiphilic block copolymers represent artificial systems designed to mimic natural membranes but with improved stability and robustness to support applications in domains such as medicine and technology. Here we present methods to produce, characterize, and modify membranes based on amphiphilic block copolymers, with appropriate properties in order to combine them with biomolecules (enzymes, proteins, DNA) in a biomimetic strategy. We indicate both the advantages of using these membranes and the limitations one can encounter when working with planar membranes. While still in its early stage of research, development of planar artificial membranes decorated with biomolecules represents a novel strategy with high potential for valuable nanometer scale applications.



There are significant efforts today to design synthetic membranes, as mimics of biomembranes with improved properties for applications in medicine, catalysis, environmental sciences, and technology.[1]

In this respect, amphiphilic block copolymers represent ideal candidates to generate membranes because they self-assemble in dilute aqueous solutions, and generate different architectures such as spherical, cylindrical, and lamellar assemblies.[2] During the last two decades, biomimetic polymer membranes, formed at the air−water interface, and more recently polymer vesicles have been intensively developed and investigated.[3,4] On the contrary, planar solid-supported membranes based on amphiphilic block copolymers represent an emerging area with only few successful examples, which we will include below.

Both vesicular and planar membranes can be generated by self-assembly of an amphiphilic copolymer, irrespective of the chemical nature of the blocks, if it is appropriately designed in terms of molecular properties. The chemical nature and length of each polymer block serve to modulate vesicular and planar membrane properties such as stability, thickness, flexibility, or permeability.[5] There are other molecular factors, which specifically influence the behavior of particular membrane architecture. For example, the hydrophilic-to-hydrophobic ratio is a crucial factor governing the generation of vesicles, while it does not influence the formation of planar membranes. In solution, polymer vesicles are more stable than free-standing membranes, which makes them ideal candidates to replace lipidic vesicles for drug delivery, or even for development of artificial organelles.[6] Interestingly, solid-supported membranes generally have higher stability than polymer vesicles and planar freestanding membranes; the solid support allows them to preserve their structure even after drying.[7] On the other hand, in polymer vesicles and free-standing membranes, the membrane is hydrated from both sides, while solid-supported membranes are asymmetric. This difference requires specific strategies when biomolecules have to be inserted/attached in/to a polymer membrane that should cope with the intrinsic symmetry of the membrane.

An interesting approach to develop planar membranes is to preserve a bilayer structure that is specific to lipid membranes of cells while introducing new properties or functionality. A smart strategy to introduce functionality to membranes based on amphiphilic block copolymers is to go one step further in mimicking cell membranes, and introduce biomolecules (proteins, enzymes, DNA, etc.) as active compounds. They allow transport of ions/molecules through the membrane support, facilitate signaling processes, or serve to sense changes in the environment of the membrane or inside it. However, the combination of membranes based on amphiphilic block copolymers with biomolecules requires a complex scenario of conditions to preserve the structure, integrity, and activity of biomolecules in a synthetic environment. For example, preparative methods for generating biodecorated membranes should avoid the use of organic solvents or pH conditions in which the biomolecule denatures.[8,9] In addition, the synthetic membrane must possess specific properties supporting their match with biomolecules, such as thickness, internal structure, or fluidity.[10,11] Specific steps are necessary to build biomimetic membranes based on amphiphilic block copolymers that accommodate biomolecules for new properties and functionality. These steps are discussed in this viewpoint, together with further developments that are expected to expand both the combinations possible and their applications.

Various amphiphilic copolymers are synthesized starting from a broad range of monomers and by a variety of

Received: November 14, 2013
Accepted: December 24, 2013
Published: December 27, 2013

techniques, such as living anionic and cationic polymerization,[12−14] controlled radical polymerization,[15,16] ring-opening polymerization,[17] or "click" chemistry.[18,19] Desired properties are introduced by (i) modulation of hydrophilic/hydrophobic blocks, block lengths and length ratios, (ii) functionalization of one polymer block,[20] (iii) selection of stimuli-responsive polymer blocks,[21,22] and (iv) use of biocompatible and biodegradable polymer blocks when medical applications are intended.[23] For example, the end groups of block copolymers are functionalized[20] by introduction of molecules such as biotin (for recognition and immobilization),[24] methacrylate (for stabilization by cross-linking),[25] dyes (for imaging),[24] and drugs (for drug delivery).[26]

However, the appropriate interaction between the polymer membrane and biological molecules is a critical requirement, which limits the number of possible copolymers that can be used for insertion/attachment of biomolecules in/to the synthetic membrane. Thus, the composition of the polymer layer acts as key factor for a successful combination of a biomimetic membrane with biomolecules. Self-assembly of amphiphilic block copolymers is the driving force that supports formation of synthetic membranes in a variety of situations: membranes in solution, free-standing membranes, monolayers at air−water interface, and solid-supported membranes (Figure 1).[27]

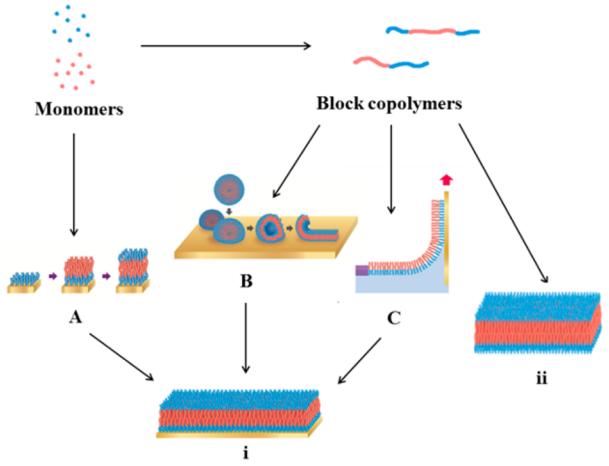


Figure 1. Schematic representation of solid-supported (i) and free-standing (ii) planar polymer membranes. Surface-immobilized membranes are prepared by surface-initiated polymerization (A), vesicle spreading and fusion (B), and monolayer transfer (C).

The methods reported for physical or chemical immobilization of the polymer membranes on surfaces can be divided into two major categories: "grafting-to", and "grafting-from" methods. The "grafting-to" method involves the in-solution synthesis of polymer chains that can physically or covalently bind to the solid surface. The "grafting-from" method involves first the immobilization of initiator molecules, followed by polymerization of the monomers directly from the surface.[28] The "grafting-from" method provides good control over the brush thickness and good surface coverage, while the "grafting-to" is unable to achieve high grafting densities and membranes are not homogeneous when the deposition of the polymer chains is randomly made. In order to control the ordering and packing of the macromolecules, and thereby the thickness and architecture of the polymer layers, other types of "grafting-to" methods are used, such as spreading and fusion of micelles or vesicles on solid supports, and transfer of monolayers (e.g., Langmuir−Blodgett (LB), Langmuir−Schaefer (LS), and layer-by-layer (LbL) methods).[7,29−32] The monolayer transfer enables a homogeneous deposition over large areas and the possibility to make multilayer structures with varying layer composition, the film architectures being completely determined by the deposition sequence.

Surface modification strategies are used to induce specific properties of the membrane, such as flexibility or density, and to provide accessible functional groups for attachment of molecules.

In order to mimic cell membranes, biological molecules can be attached on polymer membrane surface or inserted within the membrane before or after the polymer membrane is created. The best known examples of biomolecules attachment/insertion, which also represent the most specialized interactions in biological systems, are (i) insertion of pore- or channel-forming peptides (biopores/channels), (ii) attachment of enzymes, and (iii) biotin-streptavidin and metal-His-tag protein couplings.[4] The modification of membranes with specific recognition sites[33−35] represents an elegant way to (i) improve their interaction with specific molecules, (ii) bind and sense the presence of specific molecules (proteins, enzymes, DNA),[36] and (iii) create new materials.[37]

A combination of methods is used to characterize planar membranes: microscopy, electrophysiological measurements, and other surface characterization tools, such as quartz crystal microbalance (QCM), surface plasmon resonance (SPR), and ellipsometry.

Microscopy techniques are fast, easy, and provide relatively straightforward specimen visualization, which reveals important features such as morphology, homogeneity, or size. Fluorescence microscopy, which allows a clear view of dye-labeled molecules/regions of the membrane, can be used for enhanced details. Fluorescence microscopy is particularly useful for investigation of planar membranes in terms of stability and dynamic processes of macromolecules, including diffusion,[38] binding constants,[37] or enzymatic reactions.[39] Scanning force microscopy (SFM) methods, such as atomic force microscopy (AFM) and scanning tunnelling microscopy (STM), are the most frequently applied techniques for determining the membrane structure on solid substrates with high resolution (within a few Å).[40] In addition, AFM serves for determination of structural defects, roughness, stability, thickness, and mechanical properties of thin films on solid substrates.[31] For example, by scratching a domain of the polybutylene-*block*-poly(ethylene oxide) (PB-*b*-PEO) bilayer, it is possible to establish the presence of the second polymer monolayer and, thus, to prove an attachment to the first layer.[7,30] Furthermore, it allows confirmation of the presence of a spacer layer between tethered bilayer membranes and the solid support.[41]

Electrophysiological measurements such as patch clamp and electrochemical impedance spectroscopy (EIS) are used to characterize the incorporation of proteins because membranes based on amphiphilic block copolymers, which are usually good insulators,[42] become conductive when enriched with carriers, pores, or defects. The patch clamp technique permits monomolecular resolution investigation on transmembrane channels. Microstructured planar chip devices for patch clamp measurement allow the characterization of polymer membranes that have painted or folded bilayers,[43−45] or bilayers produced

dx.doi.org/10.1021/mz400590c | *ACS Macro Lett.* 2014, 3, 59−63

from the fusion of giant unilamellar vesicles (GUVs) on apertures in a chip.[46,47]

EIS measurements characterize the electrochemical properties of large area polymer membranes in both a qualitative and quantitative way. For example, EIS establishes the influence of different cations and components on membrane properties,[48] and the proton flux across bilayers membranes induced by free fatty acids.[49] By comparing the electrical conductance of a tethered solid-supported polymer bilayer membrane (TSSPBM) before and after incubating with a polypeptide (α-hemolysin), it was proved that TSSPBM has the flexibility for incorporating membrane proteins.[50] The disadvantage of electrophysiological measurements like EIS is that the membranes must be specially prepared to fit the sample cell and carefully handled throughout the process.

Other surface characterization tools such as SPR, QCM, and ellipsometry are applied for characterization of planar membranes in terms of membrane thickness, in situ bilayer formation, and insertion/attachment of biomolecules.[7,51] In addition, SPR and ellipsometry are used to characterize the optical properties of membranes, while QCM allows an estimation of the surface coverage/grafting density.

In solution there are two possibilities to generate membranes: monolayers at the air−water interface and planar freestanding membranes.

Monolayers at the air−water interface are the simplest models of the biological membranes used for investigation of interactions with biomolecules in different conditions, for example, temperature, pH of the subphase and surface pressure of the film, and thus in different physical states of the film (Figure 2).[52] Langmuir monolayer technique is broadly used to study synthetic films of polymers,[53−55] mixtures of polymers,[56] and mixtures of polymers with lipids[57,58] or with biomolecules.[59] In addition, polymer films at air−water interface are used as scaffolds in biomineralization to control the growth of inorganic particles.[60−62]

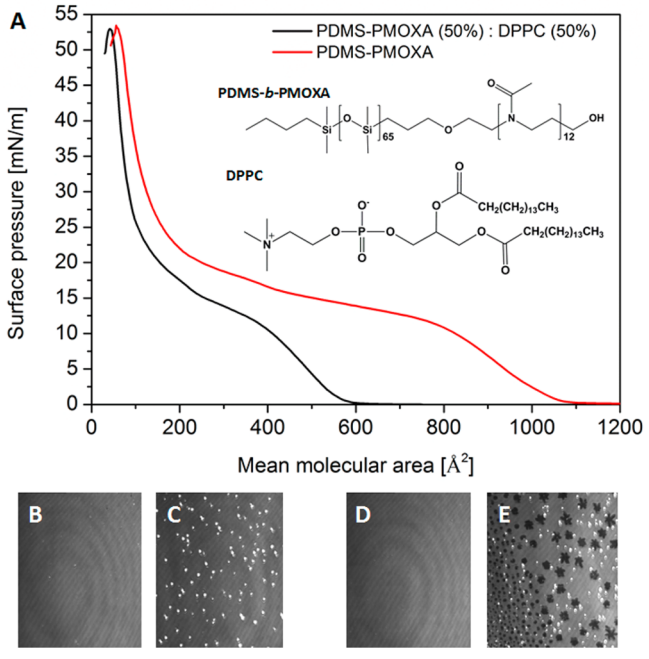


Figure 2. Surface pressure−area isotherms of pure PDMS-*b*-PMOXA diblock copolymer (in red) and mixture of PDMS-*b*-PMOXA and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC; molar ratio 0.5−0.5, in black) (A). Brewster angle microscopy images of monolayers at the air−water interface from PDMS-*b*-PMOXA, at 20 mN m^{-1} (B) and 50 mN m^{-1} (C), and PDMS-*b*-PMOXA−DPPC at 20 mN m^{-1} (D) and 50 mN m^{-1} (E).

A critical point when working with Langmuir monolayers is the necessity of a high precision, as any impurities in the monolayer material or subphase can substantially influence the results. Other limiting factors can be (i) leakage of the barriers, (ii) subphase evaporation, (iii) solubility of monolayer material, and (iv) spreading of different amounts of material.[52] Due to their low stability plus the lack of appropriate methods of characterization, Langmuir monolayers are usually transferred to solid-substrates by LB and LS techniques, to enable characterization in more details. Examination of monolayers allows understanding the interactions and behavior of molecules in a particular synthetic environment, which supports the combination of artificial membranes with biomolecules.[62]

For planar freestanding membranes both sides of the membrane are accessible, which allows mimicking a cell membrane in physiological conditions.[63] The insertion of a channel protein in such freestanding membranes is usually characterized by the change in the conductance of the system.[64]

While planar freestanding lipid membranes (known as Black Lipid Membranes) have been widely used for investigations of protein insertion mechanisms, only very few examples of freestanding membranes based on amphiphilic block copolymers, have been reported for investigation of protein insertion.[65] The first example of such a synthetic freestanding membrane was based on poly(2-methyloxazoline)-*block*-poly(dimethylsiloxane)-*block*-poly(2-methyloxazoline) (PMOXA-*b*-PDMS-*b*-PMOXA) copolymer, which formed a planar membrane with thickness of 10 nm and a surface area up to 1 mm^2. Transmembrane proteins were successfully incorporated and kept their functions in these polymer membranes.[66,67]

A major disadvantage of planar freestanding membranes is their low stability due to the limited lateral tension, which may lead to immediate membrane rupture.[68] Because of this instability as well as difficulty in handling, planar freestanding membranes are of little technological interest. Their applications are limited to basic studies of membrane interactions with biomolecules. To improve this type of synthetic membrane, pore-solid-supported membranes have been introduced, because they preserve favorable properties of free-standing membranes, while being more stable and easier to handle.

Planar solid-supported membranes are obtained by attaching them to a solid surface, resulting in an improved mechanical stability compared to isolated free-standing membranes.[69] They can be advantageously modified to attach/insert active compounds, in order to generate "smart surfaces" with a desired functionality.[4]

The increased thickness of membranes based on amphiphilic block copolymers, 3−40 nm compared with the 1−3 nm hydrophilic layer of lipid membranes,[70] prevents the strong interactions and frictional coupling between solid substrate and incorporated proteins that could result in partial loss of functionality or complete protein denaturation.[71] Moreover, in a planar solid-supported polymer membrane, the noncovalent interactions between two polymer layers allow a certain degree of membrane fluidity, essential for the insertion of peptides and membrane proteins.[30,50] Membrane proteins are inserted either during the membrane formation process or after the membrane is formed. As a first example, α-hemolysin (αHL) has been successfully reconstituted in an amphiphilic PB-*b*-PEO solid-

dx.doi.org/10.1021/mz400590c | *ACS Macro Lett.* 2014, 3, 59−63

supported membrane by voltage destabilization of the membrane (Figure 3a).[50]

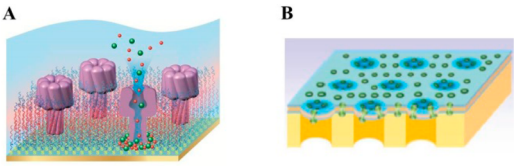


Figure 3. Schemes of membrane protein constituted (A) solid-supported planar membranes, (B) pore-solid-supported membranes.

Pore-solid-supported membranes represent a step further in synthetic membrane development because they combine the mechanical stability of solid-supported membranes with the advantage of free-standing membranes over the pores. This provides two accessible aqueous compartments for inserted transmembrane proteins.[72] Pore-solid-supported membranes allow the study of conformational changes in membrane proteins drawn by gradients, cargo transport, and external forces. In addition, pore-solid-supported membranes offer unprecedented mechanical stability over periods of days, with mesh sizes between 20 nm and several micrometers and in defined geometric patterns.[73] The first reported example is based on insertion of channel porin Aquaporin (AqpZ) in pore-solid-supported membranes, to generate a highly permeable membrane for small solutes such as ions, nutrients, or antibiotics (Figure 3b).[74,75]

Perspectives: Membranes based on amphiphilic block copolymers are known to be more stable than lipid membranes and can be expected to serve as mimics of biomembranes with improved properties if appropriately designed. Various methods are used to produce planar amphiphilic copolymer membranes as free-standing films in solution, as monolayers at an air−water interface, or as surfaces in planar solid-supported or pore-solid-supported membranes. Although copolymer membranes in solution or at an air−water interface possess the flexibility to allow insertion of biomolecules, they have low stability and are difficult to characterize. Therefore, they represent only model systems and until now have no technological application.

By contrast, solid-supported planar membranes are stable and allow insertion/attachment of biomolecules under appropriate conditions; however, there is no single general strategy to obtain planar biomimetic membranes based on amphiphilic block copolymers. The appropriate approach is to develop a specific type of membrane for a particular application by tuning the membrane properties (flexibility, thickness, homogeneity) and conditions (preparation method, solvents) to cope with the challenges of the desired application. A drawback of solid-supported membranes is to not allow investigations that mimic physiological conditions, such as the transport of matter or the mechanism of ion flux through a membrane. A clever approach to counteract this drawback is to use pore-solid-supported membranes for insertion of proteins and to study transport processes. The main critical point in this particular case is to determine which methods can successfully accommodate functional membrane protein insertion.

One of the main challenges in the development of planar synthetic biomembranes is to scale up their effective area, while preserving the properties, in order to support industrial applications. Although significant efforts have been made to improve the stability, robustness, and lifetime of polymer membranes, there are still various methods, as for example membrane cross-linking, which have been investigated to improve membrane properties.

Moreover, approaches to support simultaneous reconstitution of various membrane proteins in the membrane and subsequent attachment of different biomolecules are still required to generate multifunctional surfaces. Multifunctional surfaces are expected to offer a rapid and complete reply to complex situations, as for example simultaneous biosensing of multiple compounds. Besides, the development of mixed films/membranes based on copolymers or combinations of copolymers and lipids have still to be explored for modulation of membrane properties, such as thickness, flexibility, and interaction of biomolecules.

A not yet explored step in the development of biomimetic membranes based on amphiphilic block copolymers is the design of stimuli-responsive membranes. Stimuli such as temperature, light, pH, and the presence of ions or molecules can induce important membrane conformational changes, which may be used in the development of biosensors or for release of a specific molecule "on demand". In addition, dual stimuli-responsive block copolymers can provide multiresponsiveness of the membrane, which will support simultaneous functions or applications.

AUTHOR INFORMATION

Corresponding Author

*E-mail: cornelia.palivan@unibas.ch; wolfgang.meier@unibas.ch.

Author Contributions

†These authors contributed equally to this work (J.K. and X.Z.).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The work was financially supported by Swiss National Science Foundation, Swiss Nanoscience Institute, NRP 62 "Smart Materials" and University of Basel.

REFERENCES

(1) Nielsen, C. *Anal. Bioanal. Chem.* **2009**, *395*, 697.
(2) Ramanathan, M.; Shrestha, L. K.; Mori, T.; Ji, Q.; Hill, J. P.; Ariga, K. *Phys. Chem. Chem. Phys.* **2013**, *15*, 10580.
(3) Discher, D. E.; Eisenberg, A. *Science* **2002**, *297*, 967.
(4) Zhang, X. Y.; Tanner, P.; Graff, A.; Palivan, C. G.; Meier, W. *J. Polym. Sci., Part A: Polym. Chem.* **2012**, *50*, 2293.
(5) Discher, D. E.; Eisenberg, A. *Science* **2002**, *297*, 967.
(6) Najer, A.; Wu, D. L.; Vasquez, D.; Palivan, C. G.; Meier, W. *Nanomedicine* **2013**, *8*, 425.
(7) Belegrinou, S.; Dorn, J.; Kreiter, M.; Kita-Tokarczyk, K.; Sinner, E.-K.; Meier, W. *Soft Matter* **2010**, *6*, 179.
(8) Kita-Tokarczyk, K.; Grumelard, J.; Haefele, T.; Meier, W. *Polymer* **2005**, *46*, 3540.
(9) Meng, F.; Hiemstra, C.; Engbers, G. H.; Feijen, J. *Macromolecules* **2003**, *36*, 3004.
(10) Naumann, C. A.; Prucker, O.; Lehmann, T.; Rühe, J.; Knoll, W.; Frank, C. W. *Biomacromolecules* **2001**, *3*, 27.
(11) Wagner, M. L.; Tamm, L. K. *Biophys. J.* **2000**, *79*, 1400.
(12) Faust, R. In *Polymer Science: A Comprehensive Reference*; Matyjaszewski, K., Möller, M., Eds.; Elsevier: Amsterdam, 2012; p 501.
(13) Ishizone, T.; Sugiyama, K.; Hirao, A. In *Polymer Science: A Comprehensive Reference*; Matyjaszewski, K., Möller, M., Eds.; Elsevier: Amsterdam, 2012; p 591.

(14) Quirk, R. P. In *Polymer Science: A Comprehensive Reference*; Matyjaszewski, K., Möller, M., Eds.; Elsevier: Amsterdam, 2012; p 559.
(15) Braunecker, W. A.; Matyjaszewski, K. *Prog. Polym. Sci.* **2007**, *32*, 93.
(16) Malinova, V.; Belegrinou, S.; Bruyn Ouboter, D.; Meier, W. In *Organic Electronics*; Grasser, T., Meller, G., Li, L., Eds.; Springer: Berlin, Heidelberg: 2010; Vol. *223*, p 213.
(17) Kobayashi, S. In *Polymer Science: A Comprehensive Reference*; Matyjaszewski, K., Möller, M., Eds.; Elsevier: Amsterdam, 2012; p 397.
(18) van Dijk, M.; Rijkers, D. T. S.; Liskamp, R. M. J.; van Nostrum, C. F.; Hennink, W. E. *Bioconjugate Chem.* **2009**, *20*, 2001.
(19) Xiong, X.-B.; Binkhathlan, Z.; Molavi, O.; Lavasanifar, A. *Acta Biomater.* **2012**, *8*, 2017.
(20) Pawar, P. V.; Gohil, S. V.; Jain, J. P.; Kumar, N. *Polym. Chem.* **2013**, *4*, 3160.
(21) Li, M.-H.; Keller, P. *Soft Matter* **2009**, *5*, 927.
(22) Schacher, F.; Ulbricht, M.; Müller, A. H. E. *Adv. Funct. Mater.* **2009**, *19*, 1040.
(23) Li, S.; Meng, F.; Wang, Z.; Zhong, Y.; Zheng, M.; Liu, H.; Zhong, Z. *Eur. J. Pharm. Biopharm.* **2012**, *82*, 103.
(24) Grzelakowski, M.; Onaca, O.; Rigler, P.; Kumar, M.; Meier, W. *Small* **2009**, *5*, 2545.
(25) Nardin, C.; Hirt, T.; Leukel, J.; Meier, W. *Langmuir* **2000**, *16*, 1035.
(26) Peer, D.; Karp, J. M.; Hong, S.; FarokHzad, O. C.; Margalit, R.; Langer, R. *Nat. Nanotechnol.* **2007**, *2*, 751.
(27) Belegrinou, S.; Menon, S.; Dobrunz, D.; Meier, W. *Soft Matter* **2011**, *7*, 2202.
(28) Barbey, R.; Lavanant, L.; Paripovic, D.; Schüwer, N.; Sugnaux, C.; Tugulu, S.; Klok, H.-A. *Chem. Rev.* **2009**, *109*, 5437.
(29) Decher, G. *Science* **1997**, *277*, 1232.
(30) Dorn, J.; Belegrinou, S.; Kreiter, M.; Sinner, E.-K.; Meier, W. *Macromol. Biosci.* **2011**, *11*, 514.
(31) Rakhmatullina, E.; Mantion, A.; Bürgi, T.; Malinova, V.; Meier, W. *J. Polym. Sci., Part A: Polym. Chem.* **2009**, *47*, 1.
(32) Rakhmatullina, E.; Meier, W. *Langmuir* **2008**, *24*, 6254.
(33) Boukobza, E.; Sonnenfeld, A.; Haran, G. *J. Phys. Chem. B* **2001**, *105*, 12165.
(34) Pfeiffer, I.; Höök, F. *J. Am. Chem. Soc.* **2004**, *126*, 10224.
(35) Yoshina-Ishii, C.; Miller, G. P.; Kraft, M. L.; Kool, E. T.; Boxer, S. G. *J. Am. Chem. Soc.* **2005**, *127*, 1356.
(36) Nehring, R.; Palivan, C. G.; Casse, O.; Tanner, P.; Tüxen, J.; Meier, W. *Langmuir* **2009**, *25*, 1122.
(37) Egli, S.; Nussbaumer, M. G.; Balasubramanian, V.; Chami, M.; Bruns, N.; Palivan, C.; Meier, W. *J. Am. Chem. Soc.* **2011**, *133*, 4476.
(38) Jaskiewicz, K.; Larsen, A.; Schaeffel, D.; Koynov, K.; Lieberwirth, I.; Fytas, G.; Landfester, K.; Kroeger, A. *ACS Nano* **2012**, *6*, 7254.
(39) Tanner, P.; Onaca, O.; Balasubramanian, V.; Meier, W.; Palivan, C. G. *Chemistry* **2011**, *17*, 4552.
(40) Binnig, G.; Quate, C. F.; Gerber, C. *Phys. Rev. Lett.* **1986**, *56*, 930.
(41) Wang, H.; Chung, T.-S.; Tong, Y. W.; Meier, W.; Chen, Z.; Hong, M.; Jeyaseelan, K.; Armugam, A. *Soft Matter* **2011**, *7*, 7274.
(42) Schubert, D.; Bleuel, H.; Domning, B.; Wiedner, G. *FEBS Lett.* **1977**, *74*, 47.
(43) Fertig, N.; Blick, R. H.; Behrends, J. C. *Biophys. J.* **2002**, *82*, 3056.
(44) Mayer, M.; Kriebel, J. K.; Tosteson, M. T.; Whitesides, G. M. *Biophys. J.* **2003**, *85*, 2684.
(45) Pantoja, R.; Sigg, D.; Blunck, R.; Bezanilla, F.; Heath, J. R. *Biophys. J.* **2001**, *81*, 2389.
(46) Schmidt, C.; Mayer, M.; Vogel, H. *Angew. Chem., Int. Ed.* **2000**, *39*, 3137.
(47) Sondermann, M.; George, M.; Fertig, N.; Behrends, J. C. *Biochim. Biophys. Acta, Biomembr.* **2006**, *1758*, 545.
(48) Nikolov, V.; Lin, J.; Merzlyakov, M.; Hristova, K.; Searson, P. C. *Langmuir* **2007**, *23*, 13040.
(49) Arcisio-Miranda, M.; Abdulkader, F.; Brunaldi, K.; Curi, R.; Procopio, J. *Arch. Biochem. Biophys.* **2009**, *484*, 63.
(50) Zhang, X. Y.; Fu, W. Y.; Palivan, C. G.; Meier, W. *Sci. Rep.* **2013**, *3*, 2196.
(51) Marx, K. A. *Biomacromolecules* **2003**, *4*, 1099.
(52) Dynarowicz-Latka, P.; Dhanabalan, A.; Oliveira, O. N. *Adv. Colloid Interface Sci.* **2001**, *91*, 221.
(53) Cabasso, I.; Stesikova, E. *J. Phys. Chem. B* **2008**, *112*, 14379.
(54) Joncheray, T. J.; Denoncourt, K. M.; Meier, M. A. R.; Schubert, U. S.; Duran, R. S. *Langmuir* **2007**, *23*, 2423.
(55) Scheibe, P.; Barz, M.; Hemmelmann, M.; Zentel, R. *Langmuir* **2010**, *26*, 5661.
(56) Deschenes, L.; Bousmina, M.; Ritcey, A. M. *Langmuir* **2008**, *24*, 3699.
(57) Jagoda, A.; Ketikidis, P.; Zinn, M.; Meier, W.; Kita-Tokarczyk, K. *Langmuir* **2011**, *27*, 10878.
(58) Kita-Tokarczyk, K.; Itel, F.; Grzelakowski, M.; Egli, S.; Rossbach, P.; Meier, W. *Langmuir* **2009**, *25*, 9847.
(59) Haefele, T.; Kita-Tokarczyk, K.; Meier, W. *Langmuir* **2006**, *22*, 1164.
(60) Jagoda, A.; Zinn, M.; Bieler, E.; Meier, W.; Kita-Tokarczyk, K. *J. Mater. Chem. B* **2013**, *1*, 368.
(61) Junginger, M.; Kita-Tokarczyk, K.; Schuster, T.; Reiche, J.; Schacher, F.; Müller, A. H. E.; Cölfen, H.; Taubert, A. *Macromol. Biosci.* **2010**, *10*, 1084.
(62) Kita-Tokarczyk, K.; Meier, W. *Chimia* **2008**, *62*, 820.
(63) Ries, R. S.; Choi, H.; Blunck, R.; Bezanilla, F.; Heath, J. R. *J. Phys. Chem. B* **2004**, *108*, 16040.
(64) Van Gelder, P.; Dumas, F.; Winterhalter, M. *Biophys. Chem.* **2000**, *85*, 153.
(65) Nardin, C.; Winterhalter, M.; Meier, W. *Langmuir* **2000**, *16*, 7708.
(66) Meier, W.; Nardin, C.; Winterhalter, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 4599.
(67) Wong, D.; Jeon, T. J.; Schmidt, J. *Nanotechnology* **2006**, *17*, 3710.
(68) Winterhalter, M. *Curr. Opin. Colloid Interface Sci.* **2000**, *5*, 250.
(69) Senaratne, W.; Andruzzi, L.; Ober, C. K. *Biomacromolecules* **2005**, *6*, 2427.
(70) Tanaka, M.; Sackmann, E. *Nature* **2005**, *437*, 656.
(71) Sinner, E.-K.; Knoll, W. *Curr. Opin. Chem. Biol.* **2001**, *5*, 705.
(72) Lazzara, T. D.; Carnarius, C.; Kocun, M.; Janshoff, A.; Steinem, C. *ACS Nano* **2011**, *5*, 6935.
(73) Mey, I.; Stephan, M.; Schmitt, E. K.; Müller, M. M.; Ben Amar, M.; Steinem, C.; Janshoff, A. *J. Am. Chem. Soc.* **2009**, *131*, 7031.
(74) Kumar, M.; Habel, J. E. O.; Shen, Y.-x.; Meier, W. P.; Walz, T. *J. Am. Chem. Soc.* **2012**, *134*, 18631.
(75) Wang, H.; Chung, T.-S.; Tong, Y. W.; Jeyaseelan, K.; Armugam, A.; Chen, Z.; Hong, M.; Meier, W. *Small* **2012**, *8*, 1185.